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Antibodies to *Pityrosporum orbiculare* in Patients with Tinea Versicolor and Controls of Various Ages

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Sera from patients with tinea versicolor and controls of various ages were investigated with the indirect immunofluorescence technique for antibodies against *Pityrosporum orbiculare*, the etiologic agent of tinea versicolor. No differences in titers were observed between patients and adult controls. Also, there were no differences in antibody titers in the patient group with regard to age and sex, or to duration and distribution of lesions. A statistically significant difference in antibody titers was observed between adult controls and children, particularly the youngest.

Antibodies against *Candida albicans* from randomly selected sera from the same groups showed the same tendency, although only statistically significant when children of 5 years or younger were compared with adult controls.

This investigation indicates that although *P. orbiculare* is capable of inducing antibodies, these are not correlated to tinea versicolor but occur when an individual becomes colonized with the organism.

Pityrosporum orbiculare is a member of the normal human cutaneous flora [1–3], and also the etiologic agent of tinea versicolor [4,5]. *P. ovale*, another member of the genus *Pityrosporum*, is probably identical to *P. orbiculare* [4–7]. *P. orbiculare* (*P. ovale*) can be cultured from the normal skin in the majority of adults [1–3,8], but usually not from newborn or infants [8].

In 1961, Sternberg and Keddie described antibodies against *P. orbiculare* in 2 patients with rapidly spreading tinea versicolor [9]. In 1967, using the indirect immunofluorescent (IIF) technique Alexander found antibodies against both *P. orbiculare* and *P. ovale* in patients with dandruff [10]. In a recent report comparing patients with tinea versicolor and patients with chronic mucocutaneous candidiasis, antibodies against *P. orbiculare* were found in both patients with tinea versicolor and controls [11]. Antibody titers were higher in patients compared to controls [11]. Sohnle and Collins-Lech had earlier reported that cell-mediated immunity against *P. orbiculare* is defective in patients with tinea versicolor [12].

The aim of the present study was to compare antibody titers against *P. orbiculare* in patients with tinea versicolor and controls of various ages.

MATERIALS AND METHODS

Characteristics of Patients

We investigated antibodies against *P. orbiculare* in 30 patients with tinea versicolor. The diagnosis was confirmed clinically with Wood's light, and microscopically. They were also studied with regard to age and sex distribution and with regard to duration and distrubution of tinea versicolor lesions.

Characteristics of Controls

Fifteen patients with a mild eczema of the hands and 6 healthy volunteers were included, all of these had never had tinea versicolor. Six umbilical cord sera and, as a control, sera from their mothers were investigated. To look for differences in antibody titers among ages, six-month-old (n = 8), 5-year-old (n = 8), 10-year-old, (n = 10), and 15-year-old (n = 10) children were studied. Among the children six months of age, 2 had a cerebral tumor, 1 congenital heart disease, 1 epilepsy, 1 was a healthy control, and 3 had acute infectious diseases. The other control children consisted of 11 with epilepsy and 17 with food allergy; these 28 patients were all ambulatory.

IIF-Technique on Sera from Patients with Tinea Versicolor and Controls

Cells of *P. orbiculare*, ATCC No. 42132, were used as the antigen. For controls, all sera from patients and adult controls and 6 sera from each group of children at the age of 5, 10, and 15 years were also incubated with cells of *Saccharomyces steineri* from our own collection. Six sera from each of the groups of adults and children, and the 6 umbilical cord sera were also incubated with cells of *Candida albicans*, 207A, originally obtained from Dr. H. F. Hasenclever (National Institutes of Health, Bethesda). In addition, sera from 6 patients with tinea versicolor and 6 adult controls were also studied for antibodies against *P. ovale*, ATCC No. 1452. *P. orbiculare* and *P. ovale* were grown at 37°C for 3 days on a medium containing olive oil, glycerol monostearate, and Tween 80. The medium has been described in detail [6]. *C. albicans* and *S. steineri* were grown at 37°C on Sabouraud's agar.

Fungal cells, in a concentration of 10^6 cells ml^{-1}, were washed in phosphate-buffered saline (PBS), pH 7.2, smeared on nonfluorescent 10-hole Cooke's glass slides, 10 μl in each hole, air dried, and stored at −20°C until used. The cells were incubated with 25-μl portions of sera diluted 2-fold in PBS containing 4% bovine serum albumin (BSA), starting dilution 1 in 10, in a moist chamber at room temperature for 20 min. The slides were then washed in PBS. In a second step the slides were incubated with fluorescein isothiocyanate (FITC)-labeled sheep antihuman Ig (National Bacteriological Laboratory, Stockholm, Sweden, lot SH 07 A 31, molar F/p ratio 3.4, protein concentration 5.7 mg ml^{-1}), 25 μl in each hole, and diluted 1 in 20 for another 20 min. The working dilution was determined by chessboard titration. After several washes in PBS, pH 7.2, the preparations were mounted in glycerol with 10% PBS pH 7.2, and examinated in a Zeiss fluorescence microscope with incident light and equipped with a HBO-200 W/4 lamp. In all sera investigated, endpoints were the highest dilution of sera where clear rings of fluorescence surrounding cells still appeared. When the sera were investigated their origins were unknown to the reader.

Methodologic controls for the study were as follows: (1) the yeast cells alone; (2) cells incubated with antiserum alone; (3) cells incubated with FITC-conjugated antihuman Ig alone; (4) known positive sera.

Statistics

The Student's *T*-test for unpaired samples was used to compare the groups [13]. The level of significance was taken as $p < 0.02$.

RESULTS

The 30 patients with tinea versicolor included 20 females and 10 males with a mean age of 34 years. Duration of tinea versicolor varied between 6 months and 11 years, mean 2.5 years. Most of the patients had nummular lesions on the upper trunk, but 7 had extensive lesions, with more than half of the trunk skin involved. The adult controls were 15 females and 7 males, mean age 39 years. The mean antibody titers, against *P.*

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Abbreviations:
BSA: bovine serum albumin
FITC: fluorescein isothiocyanate
IFF: indirect immunofluorescent
PBS: phosphate-buffered saline

TABLE I. *Indirect immunofluorescence on* Pityrosporum orbiculare *and sera from tinea versicolor patients and controls of various ages*

Group	Anti-*P. orbiculare*	
	Mean titer ± SD	Significance[a]
1. Adult controls (n = 21)	276.19 ± 227.30	
2. Tinea versicolor (n = 30)	279.33 ± 278.94	$p > 0.5$
3. Umbilical cord blood (n = 6)	86.67 ± 58.88	$p > 0.05$
4. Mothers, controls to group No. 3 (n = 6)	160.00 ± 87.64	$p > 0.05$
5. Six-month-old children (n = 8)	5.00 ± 5.35	$p < 0.01$
6. Five-year-old children (n = 8)	28.75 ± 12.46	$p < 0.01$
7. Ten-year-old children (n = 10)	42.00 ± 22.01	$p < 0.01$
8. Fifteen-year-old children (n = 10)	60.00 ± 21.08	$p < 0.01$

[a] Group No. 1 was compared with Nos. 2–7 using Student's *t*-test.

TABLE II. *Indirect immunofluorescence on* Candida albicans *and sera from tinea versicolor patients and controls of various ages*

Group	Anti-*C albicans*	
	Mean titers ± SD	Significance[a]
1. Adult controls (n = 6)	106.67 ± 20.66	
2. Tinea versicolor (n = 6)	66.67 ± 60.22	$p > 0.5$
3. Umbilical cord blood (n = 6)	70.00 ± 50.20	$p > 0.5$
4. Six-month-old children (n = 6)	1.67 ± 4.08	$p < 0.01$
5. Five-year-old children (n = 6)	11.67 ± 4.08	$p < 0.01$
6. Ten-year-old children (n = 6)	35.00 ± 12.25	$p < 0.02$
7. Fifteen-year-old children (n = 6)	56.67 ± 26.58	$p > 0.05$

[a] Group No. 1 was compared with Nos. 2–7 using Student's *t*-test.

orbiculare, of sera from patients with tinea versicolor and controls of various age groups are shown in Table I. The antibody titers in patients with tinea versicolor were not correlated to extension or duration of lesions or the age of the patients. The mean antibody titer in sera from the 7 patients with extensive lesions was 251.43 compared to 279.33 for all patients. The mean antibody titer in sera from 16 patients with tinea versicolor of more than two years' duration was 266.25 compared to 297.29 for 14 patients with a shorter duration of the disease. The mean antibody titer of sera from 16 patients older than the mean of 34 years was 252.86 compared to 302.50 in sera from the younger patients.

When adult control sera were compared to patient sera no statistically significant difference was observed. When adult control sera were compared to sera from children of 6 months, 5, 10, and 15 years of age, a statistically significant difference was noted, $p < 0.01$. There was no statistically significant difference in antibody titers against *P. orbiculare* between sera from adult controls and umbilical cord sera. The antibody titers in the control group of mothers were higher than in the umbilical cord sera, but here again no statistically significant difference was seen. Children with epilepsy or food allergy were randomly distributed among the different age groups. No statistically significant difference was observed between antibody titers to *P. orbiculare* from children with epilepsy compared to children with food allergy.

Table II shows the mean antibody titers against *C. albicans* of sera from patients and controls. The groups are small in number but there is a tendency toward the same distribution as for antibodies against *P. orbiculare*—statistically significant when sera from adult controls were compared to sera from children at the age of 6 months ($p < 0.01$), 5 years ($p < 0.01$), and 10 years ($p < 0.02$).

A 10-year old boy with food allergy had a titer of 40 against *S. steineri*, otherwise all sera tested against this organism were negative. The 6 patient and 6 adult control sera investigated for antibodies against *P. ovale* had the same titers as for *P. orbiculare*. The methodologic controls 1–3 were negative.

DISCUSSION

In this investigation no statistically significant difference was found in antibody titers against *P. orbiculare* in patients with tinea versicolor as compared to adult controls. Also, there was no difference correlated to duration or extension of the disease. Our results are different from those of Damert et al [11] but our study includes more subjects and the level of significance in their study was lower (0.05). A defect in cell-mediated immunity in patients with tinea versicolor compared with controls, with the production of significantly less leukocyte migration inhibitory factor after stimulation with *P. orbiculare* extract, has been reported [12]. Although this defect may not explain all cases of tinea versicolor, our results indicate that humoral immunity is not involved in tinea versicolor.

We have earlier cultured *P. orbiculare* from normal-looking skin in adult controls and 15-year-old children in the same frequency as from patients with tinea versicolor [3,8]. We could not culture *P. orbiculare* in children younger than 5 years [8]. In the present investigation there was a statistically significant difference in antibody titers against *P. orbiculare* in children compared to adult controls, particularly in the youngest. In the younger groups of children only low antibody titers (< 40) were seen, and this can be compared to our earlier culture study [8]. The reason why no statistically significant difference in antibody titers against *P. orbiculare* was observed between sera from patients with tinea versicolor and adult controls and why only low titers were seen in small children may be that the production of antibodies is dependent only on colonization with the organism. *P. orbiculare* is lipophilic and the sebaceous glands mature and grow during prepuberty and puberty. This may be one explanation why *P. orbiculare* is seldom cultured from the skin of infants and, therefore, also an explanation to the low antibody titers in small children.

The antibody titers against *P. orbiculare* in the umbilical cord sera were lower than the titers in the sera of their mothers but significantly higher than serum titers from 6-month-old children. These differences are most likely explained by the transport of antibodies from the mother to the fetus across the placenta barrier.

We found the same antibody titers against *P. ovale* and *P. orbiculare*; in agreement with our earlier studies [5,6], this once again confirms the identity of these two organisms.

In one 10-year-old boy an antibody titer of 40 against *S. steineri* was found, all other sera were negative. *S. steineri* is cultured from plants [14] and not from humans, and we included it as a control in our system.

Antibody titers against *C. albicans* are often found in sera from healthy individuals, controls, and people with other diseases than candidiasis [11,15]. *C. albicans* as *P. orbiculare* is an opportunistic pathogen and is often cultured from the gastrointestinal tract or vagina in adults, more seldom in children [15]. This is in agreement with our results regarding antibody titers, where the lowest titers were found in the youngest children.

In conclusion, this study indicates that antibodies against the yeast phase of *P. orbiculare* cannot be used in the diagnosis of tinea versicolor because they are not limited to patients with this disease. It would be interesting to look at antibody titers against both the yeast and filamentous phase of *P. orbiculare*. It has been shown in tinea versicolor that when *P. orbiculare* becomes invasive it changes from its yeast to its filamentous phase [4,5]. From deep infections with *C. albicans* it is well known that there may be differences in antibody response against the yeast and filamentous phase of *C. albicans* [15,16].

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Announcements

In 1983, the *Certifying Examination of the American Board of Dermatology* will be held on October 30 and 31 in Chicago, Illinois. The deadline for receipt of applications in May 1, 1983.

The *Dermatopathology* special competence examination will be held in Chicago on November 1, 1983.

For further information on either of these examinations: Clarence S. Livingood, M.D., Executive Director, American Board of Dermatology, Henry Ford Hospital, Detroit, Michigan 48202.

The 4th CIRD Symposium—"Advances in Skin Pharmacology: Recent Methods"—will be held at Sophia Antipolis, France, on October 22–23, 1983. The Symposium will examine methods linking cell and animal experiments with clinical experience. The presentations will be divided into three sessions: New In Vitro Approaches, Recently Developed Animal Models, and Clinical Aspects. Abstracts of up to 300 words should be sent as soon as possible and not later than May 15, 1983, to: Prof. Hans Schaefer, Centre International de Recherches Dermatologiques, F-06565 Valbonne, France.

The Society for Pediatric Dermatology will hold its *Annual Educational Meeting* at Kiawah Island, South Carolina, July 21–23, 1983. For information: James E. Rasmussen, M.D., Department of Dermatology, University of Michigan Medical Center, 1405 E. Ann Street, Box 031, C-2069, Ann Arbor, Michigan 48109.

The Twenty-Fifth Annual Postgraduate Course in Dermal Pathology will be held July 31 to August 5, 1983 at The Newporter Resort, Newport Beach, California. The course is designed primarily for pathologists and dermatologists interested in cutaneous pathology. For further information: Margaret Frederick, Assistant Director, Memorial Hospital Medical Center of Long Beach–University California of Irvine Center for Health Education, 2801 Atlantic Avenue, P.O. Box 1428, Long Beach, California 90801 (213)/595–3823).